

AMENDMENTS TO THE CLAIMS

1-22. (Cancelled).

23. **(Currently Amended)** An isolated and purified glucose and fructose biopolymer comprising a 0.2 to 0.7 glucose/fructose ratio, wherein the biopolymer comprises the following properties:

- 900-1,100 Kilodalton molecular weight;
- two vitreous transition points, the first between 20°C and 30°C and the second between 190°C and 220°C;
- stability in aqueous solutions, pH values ranging from 2 to 9;
- 1,000 to 3,000 centipoise viscosity when the polymer is at 10% to 20% concentration in an aqueous solution at 30°C;
- non-hygroscopic; and
- highly soluble in water, able to form hydrogel homogeneous dispersions at maximum concentration of 50% w/v,

and wherein the biopolymer is prepared by:

a) ~~fermentation with the~~ fermenting *Lactococcus lactis* strain (NRRL B-30656) in a culture medium developed for this microorganism's growth,

b) ~~enzyme recovery by~~ recovering an extracellular enzyme extract excreted from the *Lactococcus lactis* strain (NRRL B-30656) into the culture medium by centrifuging or ultra-filtration,

c) incubating ~~metabolism products~~ the extracellular enzyme extract from ~~[[a]] the *Lactococcus lactis* strain (NRRL B-30656) (NRRLB-30656) with a sucrose substrate comprising an enzymatic extract, wherein the extracellular enzyme extract comprises or preparation having two types of glucosyltransferase and fructosyltransferase activity, thereby obtaining the biopolymer via an enzymatic reaction, and~~

d) recovering and purifying the biopolymer, and wherein hexoses in the isolated and purified biopolymer consist only of fructose and glucose.

24. (Withdrawn) A method for producing the enzymatic extract or preparation having both glucosyltransferase and fructosyltransferase activity, produced by *Lactococcus lactis* strain NRRLB-30656, which comprises:

- a) Activating the *Lactococcus lactis* NRRLB-30656r microorganism, using a medium containing sucrose as carbon source, proteins as nitrogen source and mineral salts;
- b) Fermenting the *Lactococcus lactis* NRRLB-30656 microorganism using a culture medium containing sucrose as carbon source, proteins as nitrogen source and mineral salts; and
- c) Separating the enzymatic extract or preparation from the fermented medium using centrifugation or ultrafiltration.

25. (Withdrawn) The method for producing the enzymatic extract or preparation according to claim 24, where the microorganism activating step is carried out by inoculating a medium containing sucrose as carbon source, proteins as nitrogen source and mineral salts, incubated for 10-36 hours at 25°C, with stirring at 100-400 rpm and 5 to 9 pH.

26. (Withdrawn) The method according to claim 24, where the microorganism fermenting step is carried out by cultivating the *Lactococcus lactis* NRRLB-30656 microorganism using a culture medium containing sucrose as carbon source, proteins as nitrogen Source and K_2HPO_4 , $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CaCl_2 \cdot 2H_2O$ and NaCl mineral salts, which is incubated for 12-36 hours at 25°C, with stirring at 100-400 rpm, 1-2 vvm and pH 5 to 9.

27. (Withdrawn) The method according to claim 24, where the enzymatic extract or preparation, separating step is carried out by separating the enzymatic extract or preparation from the fermented medium by centrifuging the microorganism suspension between around 3 000 to 7 000 rpm.

28. (Withdrawn) The method for producing the enzymatic extract or preparation according to claim 24, wherein in the fermentation step with the microorganism, a preinoculum with the *Lactococcus lactis* NRRLB-30656 microorganism is made using a culture medium

containing sucrose as carbon source, proteins as nitrogen source and K_2HPO_4 , $FeSO_4 \cdot 7H_2O$, $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CaCl_2 \cdot 2H_2O$ and NaCl mineral salts, and is incubated for 12-36 hours at 25°C, with stirring at 100-400 rpm, 0.1-1 vvm and pH 5 to 9.

29. (Withdrawn) The method for producing an enzymatic extract or preparation having glucosyltransferase and fructosyltransferase activity according to claim 24, wherein the sucrose concentration content as carbon source is around (10-40 g/l concentration) and proteins concentration content as nitrogen source is around 7-30 g/l and the mineral salts content is around: 7-30 g/l K_2HPO_4 , 0.01-1 g/l $FeSO_4 \cdot 7H_2O$, 0.01-0.1 g/l $MgSO_4 \cdot 7H_2O$, 0.001-0.1 g/l $MnSO_4 \cdot H_2O$, 0.001-0.01 g/l $CaCl_2 \cdot 2H_2O$ and 0.01-0.1 g/l NaCl and is incubated around 10-36 hours at 25°C, with stirring at 100-400 rpm and pH 5 to 9.

30. (Currently Amended) A method for producing an isolated and purified glucose and fructose biopolymer, comprising:

a) fermenting *Lactococcus lactis* strain (NRRL B-30656) in a culture medium,
b) recovering an extracellular enzyme extract excreted from the *Lactococcus lactis* strain (NRRL B-30656) into the culture medium by centrifuging or ultrafiltration,
c) incubating a medium comprising the extracellular enzyme extract from the *Lactococcus lactis* strain (NRRLB-30656) and a sucrose substrate, wherein the extracellular enzyme extract comprises glucosyltransferase and fructosyltransferase activity, thereby obtaining the biopolymer via an enzymatic reaction, and

d) recovering and purifying the biopolymer by precipitation or ultrafiltration

~~a) incubating metabolism products comprising an enzymatic extract or preparation from a *Lactococcus lactis* strain (NRRLB-30656) having two types of glucosyltransferase and fructosyltransferase activity obtained through fermentation, in a sucrose containing medium as carbon source, with suitable stirring speed, temperature, pH, enzymatic extract or preparation, substrate concentration and reaction time conditions for producing the biopolymer, and~~

~~b) recovering and purifying the biopolymer by precipitation or ultrafiltration,~~
wherein the biopolymer comprises the following properties:

- 900-1,100 Kilodalton molecular weight;

- two vitreous transition points, the first between 20°C and 30°C and the second between 190°C and 220°C;
- stability in aqueous solutions, pH values ranging from 2 to 9;
- 1,000 to 3,000 centipoise viscosity when the polymer is at 10% to 20% concentration in an aqueous solution at 30°C;
- non-hygroscopic; and
- highly soluble in water, able to form hydrogel homogeneous dispersions at maximum concentration of 50% w/v.

31. **(Currently Amended)** The method for producing the biopolymer, according to claim 30, wherein the incubation step further comprises enzymatic extract or preparation ~~incubation step comprises:~~

stirring the medium comprising the extracellular enzyme extract and the sucrose substrate at a speed ranging from 100-400 revolutions per minute (rpm), wherein the pH of the medium ranges from 5 to 9, the amount of extracellular enzyme extract in the medium ranges from 10-40% v/v, and the sucrose substrate concentration ranges from 5-40%; and

incubating the medium at a reaction time ranging from 12-48 hours, at a temperature ranging from 25°-35°C

~~incubating the enzymatic extract or preparation in a sucrose-containing medium as carbon source, with stirring (100-400 rpm), temperature, pH (5 to 9), enzymatic extract or preparation (10-40% v/v) and substrate concentration (5-40%) and reaction time (12-48 hours) conditions for producing the biopolymer.~~

32. **(Currently Amended)** The method according to claim 30, wherein the step of recovering and purifying the biopolymer through precipitation comprises:

- a) adding 1.2-2.0 volumes of 96% ethanol to a cold reaction mixture with stirring, wherein the quantity of added ethanol corresponds to an ethanol/reaction mixture volume, thereby obtaining a precipitated biopolymer (the quantity of added ethanol corresponds to ethanol/reaction mixture volume);

- ~~b) dissolving~~ redissolving the precipitated biopolymer obtained in step a) in half the volume of ~~deionised~~ deionized and distilled water and precipitating ~~[[it]]~~ the biopolymer obtained in step a) again with 1.2 to 2.0 volumes of an ethanol/reaction mixture volume; and
- ~~c) dissolving~~ redissolving the precipitated biopolymer in a third of the volume of water and drying through ~~lyophilisation~~ lyophilization or compressed air drying between around 50°C to 80°C until reaching around 5-6% humidity.

33. **(Currently Amended)** The method according to claim 30, wherein the step of recovering and purifying the biopolymer through ultrafiltration comprises:

ultrafiltrating ~~with the a~~ reaction mixture comprising the biopolymer ~~[[using]]~~ with a regenerated cellulose membrane having a pore size between 10,000 - 30,000 Dalton for separation by size exclusion to eliminate residual glucose and fructose and submitting the biopolymer to aspersion drying.

34. (Withdrawn) A *Lactococcus lactis* strain microorganism isolated from Colombian soil, registered under accession number NRRL B-30656.

35. (Withdrawn) The microorganism according to claim 34 which produces the enzymatic extract or preparation having both glucosyltransferase and fructosyltransferase activity.

36. (Withdrawn) The microorganism according to claim 34 which is preserved in a sucrose containing medium with 20% glycerol at -70° C and lyophilised using 10% skimmed milk.

37. **(Currently Amended)** A composition comprising the fructose and glucose biopolymer according to claim 23, wherein the composition is a viscous agent, thickener, ~~stabiliser~~ stabilizer, dispersant, film forming agent, disintegrating agent, blood plasma substitute, lubricating agent or prebiotics agent.

38. (Withdrawn) The biopolymer according to claim 23 which is used in the food industry as a thickener, viscous agent, stabiliser, dispersant, fiber and ether- and ester-based fat, oil or carbohydrate substitute.

39. (Cancelled).

40. (Previously Presented) The biopolymer according to claim 23 which is used in products obtained by extrusion for forming films apt for producing flexible and biodegradable seals and obtaining disposable biodegradable products obtained by injection or moulding and for producing flocculent agents for water treatment.

41. **(Currently Amended)** An isolated and purified glucose and fructose *Lactococcus lactis* strain (NRRLB-30656) biopolymer comprising a 0.2 to 0.7 glucose/fructose ratio,

wherein said *Lactococcus lactis* strain (NRRLB-30656) biopolymer comprises the following properties:

900-1,100 Kilodalton molecular weight;

two vitreous transition points, the first between 20°C and 30°C and the second between 190°C and 220°C;

stability in aqueous solutions, pH values ranging from 2 to 9;

1,000 to 3,000 centipoise viscosity when the polymer is at 10% to 20% concentration in an aqueous solution at 30°C;

non-hygroscopic; and

highly soluble in water, able to form hydrogel homogeneous dispersions at maximum concentration of 50% w/v, and

wherein hexoses in the isolated and purified biopolymer consist only of fructose and glucose.

42. **(Currently Amended)** An isolated and purified glucose and fructose biopolymer comprising a 0.2 to 0.7 glucose/fructose ratio,

wherein the biopolymer comprises the following properties:

- 900-1,100 Kilodalton molecular weight;

- two vitreous transition points, the first between 20°C and 30°C and the second between 190°C and 220°C;
- stability in aqueous solutions, pH values ranging from 2 to 9;
- 1,000 to 3,000 centipoise viscosity when the polymer is at 10% to 20% concentration in an aqueous solution at 30°C;
- non-hygroscopic; and
- highly soluble in water, able to form hydrogel homogeneous dispersions at maximum concentration of 50% w/v; and

and wherein the biopolymer is prepared by:

a) incubating ~~metabolism products~~ an extracellular enzyme extract from a *Lactococcus lactis* strain (NRRLB-30656) with a sucrose substrate, wherein the extracellular enzyme extract comprises ~~comprising an enzymatic extract or preparation having two types of~~ glucosyltransferase and fructosyltransferase activity, thereby obtaining the biopolymer via an enzymatic reaction, and

b) recovering and purifying the biopolymer, and
wherein hexoses in the isolated and purified biopolymer consist only of fructose and glucose.